NMR studies of folded and disordered proteins and bicelle systems

PhD dissertation

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1. Introduction

In the last decade, the essential role of IDPs in several important cellular processes has become evident and changed the structure-function paradigm [1]. Characteristically, these proteins are flexible molecules and lack a well-defined secondary or tertiary structure and due to their dynamic behavior NMR spectroscopy is a well-suited method to investigate their structure propensities at atomic resolution and to resolve the relationship between their structure, dynamics and function [2].

Protein NMR spectroscopy is a versatile tool not only in studying local environments in proteins but also in the determination of global parameters describing molecular dimensions, folding, shape and morphology of proteins and peptide-bicelle systems. Combination of NMR spectroscopy with small-angle X-ray scattering (SAXS) offers further unique advantages in structural characterizations. We investigated various systems with biological relevance: linear motifs interacting with MAPKinases are important in intracellular signaling [3]; the p53TAD-S100A4 complex plays a key role in optical biosensor development [4] and the binding might induce metastasis; membrane-protein interactions and their consequences are of high importance during the absorption of active substances/drugs through the cell membrane.

The results we obtained by studying these systems contribute significantly to the active and rapidly developing field of protein structure research.

2. Methods

Our studies proved that NMR methods can be utilized for both local and global characterization of protein and protein-bicelle structures: We utilized NMR spectroscopy methods for investigating the local structural tendencies of unlabeled MAPKinase linear motifs. We acquired homonuclear COSY, TOCSY and NOESY spectra and with high sample concentrations we performed natural abundance ^{1}H-^{15}N HSQC and ^{1}H-^{13}C HSQC experiments. We carried out SCS analysis. We also investigated the structural propensities of ^{15}N- and ^{13}C, ^{15}N-labelled p53TAD in complex with S100A4. Double labelling of the sample enabled us to collect a variety of spectra and derive several valuable parameters for structural characterization. Insufficient NOE constraints necessitated MD simulations.

We utilized PFG-NMR to determine diffusion coefficients (D)[5] and hydrodynamic radii (r_H) for the global characterization of folded, disordered and denatured proteins and protein-membrane mimetic systems (neutral and negatively charged bicelles). We studied a representative set of proteins under same experimental conditions and with optimized measurement parameters. We later combined this method with SAXS to monitor changes in global biophysical parameters - radius of gyration (r_G), r_H and r_G/r_H [6] - occurring upon bicelle-model peptide interactions.

3. Results and Discussion

3.1. MAPKAPK linear motifs

In this study we investigated the structural details of MAPK-MAPKAPK complex formation focusing on short linear motifs (NFAT, MNK1, MK2, RSK1, RSK1_S/A). We performed solution NMR-based SCS analysis on the free linear motif peptides to find how their structural propensities contribute to binding specificity.

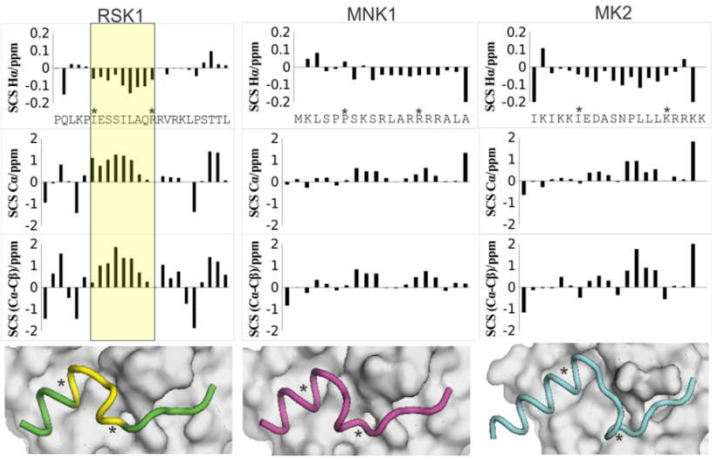

Figure 1. Structural characterization of MAPKAPK linear motifs in solution and in their MAPK bound state.

I/1. We found that all linear motif peptides are disordered in solution. We identified regions with nascent helicity and associated them with specific binding, while the lack of these regions indicated promiscuous binding. Based on these

findings structure-specificity and specificity-function relationships were established.

3.2. The p53TAD-S100A4 complex

We characterized the structure of p53TAD in complex with S100A4 using NMR spectroscopy. Based on previous p53TAD structures in complex from the literature we aimed to examine if its structural variability is preserved in complex form, focusing on the presence of dynamic fuzzy regions in the complex. The main conclusions of our study are:

II/1. The intrinsically disordered p53TAD region undergoes a disorder-to-order transition upon binding to S100A4. This result was supported by increased SSP and SCS values. p53TAD fragment in complex with S100A4 has a highly mobile long N-terminal (M1-P13) and short C-terminal (E56-P60) regions, which do not participate in complex formation. It has three rigid α-helices S20-E28, P36-P47, E51-T55 and relatively dynamic loop regions between the helices and between the first helix and the N-terminal.

II/2. The helical regions of the p53TAD are very similar in the complexes with the NCBD, MDM2, RPA, Tfb1 and S100A4 proteins, suggesting that formation of a conserved local structure is a feature of p53 recognition.

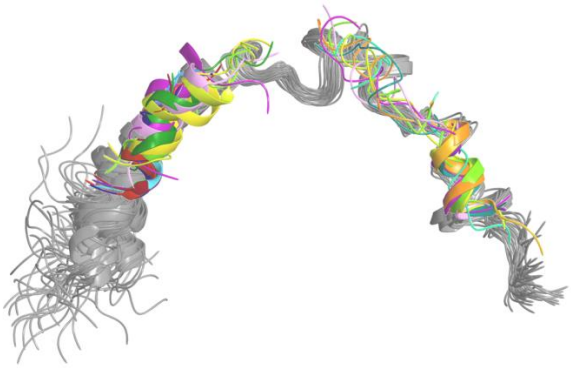

Figure 2. p53TAD regions 1-29 (TAD1), 30-47 (linker) and 48-56 (TAD2) cut from PDB structures (in color) aligned with corresponding regions of the MD simulated p53TAD-S100A4 models (grey).

3.3. A diffusion NMR based bioanalytical method to distinguish folded, disordered and denatured proteins

Central questions in the characterization of proteins are the degree of folding and the molecular dimensions which we intended to characterize by the self-diffusion coefficients (*D)* obtained from diffusion NMR measurements. We also intended to provide a simple quantitative method for estimating the debated end point of denaturation. The main conclusions of the work presented in this chapter are the following:

III/1. Based on reliable *D* values we derived empirical log*D*-log*M* relations that distinguish folded, intrinsically disordered and urea-denatured biomolecules. The established equations are easy to use analytical tools for molecular mass analysis and aggregation studies as well.

III/2. Experiments under denaturing conditions – in 8M urea solution – revealed that IDPs cannot be further denatured (decrease in local structural tendencies is not reflected in their global hydrodynamic parameters) while for folded proteins these measurements indicate whether the final unfolded state had been achieved, or if not then what was the extent of denaturation.

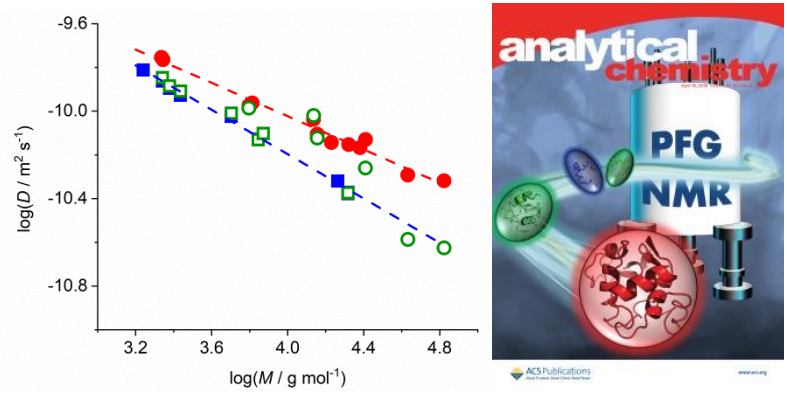

Figure 3. Logarithmic representation of diffusion coefficients as function of molecular weight for folded proteins (red circles), IDPs (blue squares), left: denatured folded proteins (open green circles) and IDPs (open green squares) viscosity corrected denatured proteins.

3.4. Bicelles and bicelle-peptide systems

Membrane-protein interactions influence biophysical properties of the membrane. We aimed to monitor them by using simplified systems: neutral and negatively charged bicelles and model peptides (surface-active melittin and transmembrane KALP23). We followed global parameters utilizing the combined NMR-SAXS approach validated for DHPC micelles. Changes in size were reflected in values r_H and r_G, while the morphology of the bicelles was characterized by parameters a, b, t_a and t_b from fitting a lenticular core-shell model on the whole SAXS scattering curve. Based on our results the following conclusions were drawn:

IV/1. To describe possible pharmaceutical drug target-membrane interactions a simple and elegant way would be the assessment of one single number for one system - namely the r_G/r_H shape factor -, however, our results show that this approach might give misleading conclusions. Instead we propose the usage of r_H and (b+t_b) values for global characterization.

IV/2. The presence of peptides alters global parameters of the bicelle: the transmembrane KALP23 induces size increase and elongation; while the mostly surface-active melittin does not significantly perturb the neutral PC bicelles. Careful data interpretation is needed for the negatively charged PC/PG bicelles, as the net charge of the peptide can have an influence as well. Thus, it is very challenging and not advisable to directly compare the results originating from different peptides. A clearer picture is obtained if comparison is done in respect to the empty bicelle system (PC or PC/PG in our case).

4. References

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4.1 Papers forming the basis of the dissertation

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4.2 Conference presentations and posters

- ’A quantitative diffusion NMR based analytical tool to distinguish folded, disordered and denatured biomolecules’ Chemistry towards biology (CTB9), Budapest, 2018, presentation
- ’ Distinction between folded and disordered proteins - A bioanalytical tool based on diffusion NMR spectroscopy’ Session of the Peptide Working Party of the Hungarian Academy of Sciences, Balatonszemes, 2018, presentation
- ’Morphological characterization of membrane mimetics by a combined NMR-SAXS approach’ MMCE Budapest, Hungary, 2017, poster
- ’Morphological characterization of membrane mimetics by a combined NMR-SAXS approach’ Session of the Peptide Working Party of the Hungarian Academy of Sciences, Balatonszemes, 2017, presentation
- ’The p53-S100A4 interaction – how can we characterize it with NMR spectroscopy?’ Session of the Hungarian NMR Working Party, Debrecen, 2016, presentation
- ’Characterization of molecular size and shape by NMR and SAXS methods’ Chianti Workshop, Grosseto , Italy, 2016, poster
- ’Protein diffusion for folded and disordered systems’ EUROMAR2015, Prague, Czech Republic, poster
- ’Protein diffusion – folded and disordered systems’ Session of the Hungarian NMR Working Party, Balatonszemes, 2015, presentation
- ’Diffusion coefficients and proteins – as seen by NMR’ Session of the Hungarian NMR Working Party, Balatonszemes, 2014, presentation